

What is claimed is:

1. A method to assay PARP activity, comprising

5 (a) contacting an immobilized PARP with NAD under conditions that allow PARP auto-ribosylation, wherein said NAD is biotinylated or avidin-conjugated;

10 (b) contacting the auto-ribosylated PARP with a detectable marker, wherein said detectable marker is avidin-conjugated in the case where the NAD of (a) is biotinylated, or wherein said detectable marker is biotinylated in the case where the NAD of (a) is avidin-conjugated, thereby forming a complex between the auto-ribosylated PARP and the detectable marker; and

(c) measuring the amount of detectable marker complexed to auto-ribosylated PARP, wherein the amount of detectable marker is indicative of the amount of PARP activity.

15 2. The method of claim 1, wherein said PARP is immobilized on a multiwell plate.

3. The method of claim 1, wherein said method is conducted at 4°C.

4. A method to assay PARP activity, comprising

20 (a) contacting an immobilized PARP with biotinylated NAD under conditions that allow PARP auto-ribosylation;

(b) contacting the auto-ribosylated PARP with an avidin-conjugated alkaline phosphatase, thereby forming a complex between the auto-ribosylated PARP and the avidin-conjugated alkaline phosphatase; and

25 (c) measuring the amount of alkaline phosphatase complexed to the auto-ribosylated PARP, wherein the amount is indicative of the amount of PARP activity.

10053262-011903  
"20220920"

5. A method to identify a modulator of PARP activity, comprising:

(a) contacting an immobilized PARP with NAD in the presence of a test agent under conditions that allow PARP autoribosylation, wherein said NAD is biotinylated or avidin-conjugated;

5 (b) contacting the auto-ribosylated PARP with a detectable marker, wherein said detectable marker is avidin-conjugated in the case where the NAD of (a) is biotinylated, or wherein said detectable marker is biotinylated in the case where the NAD of (a) is avidin-conjugated, thereby forming a complex between the auto-ribosylated PARP and the detectable marker;

10 (c) measuring the amount of detectable marker complexed to the autoribosylated PARP; and

(d) comparing the amount of detectable marker in step (c) to the amount of detectable marker in a control reaction executed without the test agent;

15 wherein an altered amount of detectable marker in the presence of the test agent relative to the amount of detectable marker in the control reaction indicates that the test agent is a modulator of PARP.

6. The method of claim 5, wherein said PARP is immobilized on a multiwell plate.

20 7. The method of claim 5, wherein said method is conducted at 4°C.

8. A method to identify a modulator of PARP activity, comprising:

(a) contacting an immobilized PARP with biotinylated NAD in the presence of a test agent under conditions that allow PARP auto-ribosylation;

25 (b) contacting the auto-ribosylated PARP with an avidin-conjugated alkaline phosphatase, thereby forming a complex between the auto-ribosylated PARP and the avidin-conjugated alkaline phosphatase;

(c) measuring the amount of alkaline phosphatase complexed to the autoribosylated PARP; and

2025-01-01 10:53:26

(d) comparing the amount of alkaline phosphatase in step (c) to the amount of alkaline phosphatase in a control reaction executed without the test agent;

wherein an altered amount of alkaline phosphatase in the presence of the test agent relative to the amount of alkaline phosphatase in the control reaction indicates that the test agent is a modulator of PARP.

9. A kit comprising:

(a) PARP immobilized on a solid support;

(b) biotinylated NAD or avidin-conjugated NAD; and

(c) an avidin-conjugated detectable marker in the case where the NAD of (b) is biotinylated, or a biotinylated detectable marker in the case where the NAD of (b) is avidin-conjugated.

10. The kit of claim 9, wherein said solid support is a multiwell plate.

11. A kit comprising:

(a) PARP immobilized on a solid support;

(b) biotinylated NAD; and

(c) avidin-conjugated alkaline phosphatase.